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Inhibition of Angiogenesis as a Strategy for Tumor Growth Control

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ABSTRACT

Angiogenesis is a complex sequence of events leading to the formation of new capillaries. Although essential to maturation and wound healing, most angiogenesis in the adult is associated with pathological events, such as the development of solid tumors. One approach to the inhibition of angiogenesis is the antagonism of basic fibroblast growth factor, a major angiogenic protein. Evidence is reviewed to suggest that inhibiting angiogenesis results in the suppression of tumor growth.

Index Entries: Angiogenesis; tumor growth; capillaries; vascularization; endothelial cells; basic fibroblast growth factor; gliomas; melanomas; suramin; platelet factor 4; minocycline; thrombospondin; fumagillin; laminin; herbimycin A; D-penicillamine.

INTRODUCTION

The concept that the inhibition of angiogenesis could result in the suppression of tumor growth was first proposed 20 yr ago by Judah Folkman (Folkman, 1971, 1972). It was a natural extension of his hypothesis that solid tumors were angiogenesis dependent. This proposal was not met with universal acceptance, however, as some researchers argued that the

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neovascularization of tumors was an inflammatory response and others claimed that tumors could form their own vascular channels lined with tumor cells. During the intervening years, supporting evidence has accumulated and the dependence of solid tumors on angiogenesis for growth is now widely accepted (Folkman, 1990a). Direct evidence also suggests that inhibition of angiogenesis is a viable approach to therapy. The purpose of this article is to review some of these studies and examine their implications for the promise of this approach.

ANGIOGENESIS

Angiogenesis or neovascularization is defined as the formation of new capillaries and is normally envisioned as a capillary with a proliferating tip, extending itself into tissue. There are critical events that must occur in order to reach that stage, starting from an intact, resting, mature capillary. The structural design of a capillary incorporates several important features that relate to the induction of angiogenesis. The wall of the capillary consists of a single, flattened endothelial cell, embedded in an extracellular matrix composed largely of proteoglycans and surrounded by a more rigid basement membrane. The whole structure seems designed to keep formed elements in, while presenting as small a barrier as possible to the diffusion of small molecules.

In order for angiogenesis to occur, cells must be stimulated to proliferate to produce the additional cells required to form the new capillary. These proliferating cells must secrete proteases, collagenases, and/or stimulatory factors to promote the degradation of the local matrix and basement membrane and escape from their containment, while maintaining the three-dimensional continuity of the capillary. The cells must also be induced to migrate down some directional signal, organize themselves into a tubule, and selectively revert back to the quiescent state to form a stable new vessel. Angiogenesis is a normal and desirable cellular process when it occurs in reproduction, development, or wound healing. However, most neovascularization in the adult is associated with pathological, abnormal capillary proliferation.

Even this oversimplified view of the induction of angiogenesis indicates clearly that the process is a complex orchestration of numerous steps that must occur in precise temporal and spatial relationships. Our knowledge of the mechanisms and signals by which these steps are induced, controlled, and terminated is incomplete, but each step in the process is a potential pharmacological control point. Any compound or biologic agent that exerts a significant effect on any single step will probably affect the overall angiogenic process as well.

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BASIC FIBROBLAST GROWTH FACTOR

Of the numerous factors and molecules reported to be angiogenic, only basic fibroblast growth factor (bFGF) has been demonstrated thus far to be a complete angiogenic signal (For a review, *see* Rifkin, 1989). This protein of about 156 amino acids has been shown to be proliferative to endothelial cells and to increase their production of plasminogen activator and other hydrolytic and proteolytic enzymes. It has chemotropic activity, as demonstrated by its ability to induce endothelial cells to migrate along its concentration gradient and to promote their organization into tubules. It is chemotrophic and can promote the survival of cells removed from their normal environment. Because of the involvement of bFGF in all of these steps of angiogenesis, it has been a major focus of interest in the development of antiangiogenic factors (Folkman, 1990b).

Early in our program, a study was conducted in nude mice bearing xenografted human DLD-2 colon carcinoma tumors to examine the possible modulation of tumor growth by human recombinant bFGF (hr-bFGF) and a neutralizing antibody to hr-bFGF. The results clearly showed that the size of the tumors in the mice that received hr-bFGF was almost double that in the control mice (Gross, in press). Not only was the size of the tumors increased, but the tumors invaded the peritoneal cavity, which was not seen in the controls. These results were particularly striking since DLD-2 cells in culture neither expressed high affinity sites for ^{125}I-hr-bFGF nor responded to the addition of hr-bFGF in the media (Gross, 1990).

Although the antibodies tested in the initial study did not show significant inhibition of tumor growth, a subsequent study using C6 rat glioma cells did demonstrate the ability of one of the antibodies, DG-2, to retard tumor growth. Unlike DLD-2 cells, C6 glioma cells express high levels of high affinity binding sites for ^{125}I-bFGF and are very responsive to hr-bFGF in culture (Gross, 1990). Likewise, two Japanese groups have demonstrated that neutralizing monoclonal antibodies against bFGF can suppress the growth of tumors from several tumorigenic cell lines expressing high affinity bFGF receptors or bFGF (Takahashi, 1991, Hori, 1991).

Autoradiographic studies of sections of various tumors with ^{125}I-bFGF demonstrated that many tumors exhibited high affinity binding, but that the labeling did not correlate with the expression of high affinity sites by the tumor cells in culture. Tumors grown from B16 murine melanoma cells or DLD-2 cells, two lines that lack high affinity sites in culture, were labeled by ^{125}I-bFGF. The same structures that were labeled were also recognized by antibodies against Factor VIII and a lectin from *Dolicus biflorus*, two putative markers for endothelial cells, suggesting that the labeling was associated with the developing vasculature (Herblin, in press).

Several molecules other than antibodies have been reported to inhibit bFGF activity and have been investigated as inhibitors of angiogenesis

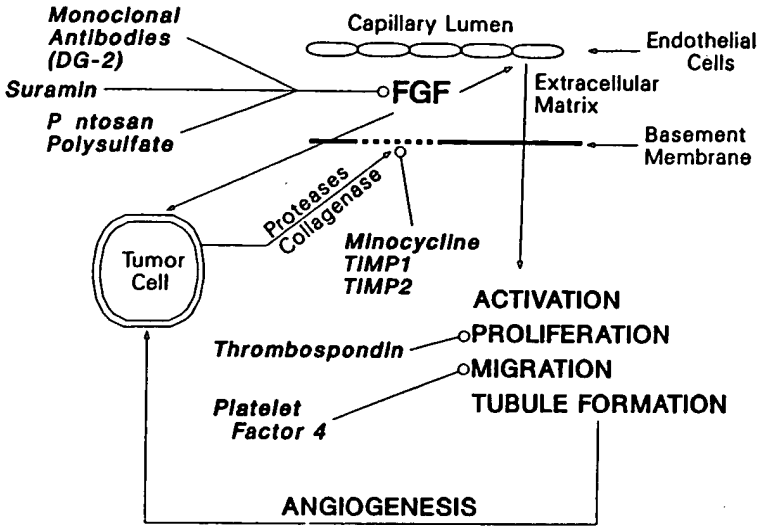

Fig. 1. The interrelationship between angiogenesis and tumor growth. Basic fibroblast growth factor occupies a central position in tumor-induced angiogenesis by virtue of its effects on both the capillary endothelial cells and many different tumor cells. Inactivation of either bFGF or its receptors should inhibit both angiogenesis and tumor growth.

and also as suppressants of tumor growth. These molecules may either mimic or bind to heparin and therefore inhibit any of the "heparin-binding" growth factors (Moses, 1991). Suramin has been shown to bind to several growth factors, including bFGF and can inhibit both angiogenesis and tumor growth (La Rocca, 1990). Similar findings have been reported for protamine (Taylor, 1982) and pentosan polysulfate (Wellstein, 1991). The general findings with these compounds has been that it is necessary to give large doses of the compounds in order to show activity and that efficacy is often limited by heparin-like toxicities.

The proposed angiogenesis loop is schematically illustrated in Fig. 1. Tumor cells secrete proteases and collagenases, and these can degrade the basement membrane and exposed extracellular matrix of a nearby capillary, releasing the trapped bFGF (Vlodavsky, 1991). Many tumor cells are responsive to bFGF and will be stimulated by the released factor. In addition, the bFGF can activate the endothelial cells of the capillary to initiate the angiogenic processes of proliferation and migration, creating new vessels to vascularize the developing tumor and supply the nutrients required for its growth. Agents that inactivate bFGF, such as suramin or neutralizing antibodies to bFGF, can be shown to inhibit both angiogenesis and tumor growth. Platelet factor 4 inhibits bFGF binding and endothelial cell migration (Sato, 1990). The angiogenic loop can also be interrupted at other sites by agents that can stabilize the basement membrane such as minocycline, believed to act through the inhibition of collagenase (Tamargo, 1991), or inhibit endothelial cell proliferation like thrombospondin (Bagavandoss, 1990).

POTENTIAL LIMITATIONS AND SOLUTIONS

The strategy of inhibiting angiogenesis to supress tumor growth is not universally accepted and there are several cogent arguments against it. Angiogenesis is an essential process in reproduction and development, as well as wound healing and the general inhibition of angiogenesis would therefore be expected to carry significant side effects. Although these considerations might limit the application of any antiangiogenic, they would not negate the benefits to be derived in those cases where it could be used safely. Some investigators feel that even if angiogenesis could be inhibited, growing tumors would be supplied by existing vessels (Folkman, 1987). Although this might occur, the process by which the tumor would gain access to the existing vessel would be essentially identical to the initial steps of angiogenesis and should also be inhibited. There are examples in which proven antiangiogenics failed to suppress tumor growth but that does not invalidate the hypothesis. It is becoming increasingly clear that no single therapy is going to be effective against all tumors and an anti-angiogenic will represent one more weapon in the physician's arsenal.

An additional possibility for avoiding unacceptable side effects stems from the complexity that is being found in the biochemistry of growth factors and their receptors. There are now at least seven members of the FGF family, including acidic and basic FGF, along with int-2, hst, KGF and others, and three classes of high affinity FGF receptors have also been reported (Baird and Klagsbrun, 1991). The predominant high affinity class includes a tyrosine kinase domain and exists as several distinct types such as flg and bek, and each type can be expressed in several forms by alternative splicing. The complexity presented by these multiple forms of both the ligand and the receptor make it difficult to sort out the precise functions mediated by the individual proteins through the various receptors, but also supports the possibility of developing specific agents that could distinguish different aspects of angiogenesis.

CONCLUSIONS

Although the focus of this review has been on bFGF and its inhibitors, several compounds have been demonstrated to be effective antiangiogenic and antitumor treatments even though they do not interfere with bFGF. Some of these are listed in Table 1 and include AGM-1470, an analog of fumagillin, that inhibits endothelial cell proliferation (Ingber, 1990) and a synthetic laminin peptide (Sakamoto, 1991) that is believed to prevent endothelial cell migration. The mechanism for these compounds is functionally, but not biochemically defined. In still other cases, even the functional basis of action is unknown. These include peptides related to platelet factor 4 (Maione, 1990, 1991), herbimycin A (Yamashita, 1989), somatostatin analogs (Woltering, 1991) and D-penicillamine with or without copper

Table 1
Compounds and Derivatives Reported to Inhibit Angiogenesis and Tumor Growth but not Basic Fibroblast Growth Factor

Compound	Proposed inhibition of	Reference
Laminin fragment	Endothelial cell migration	Sakamoto, 199
Platelet factor 4 analogs	Endothelial cell proliferation	Maione, 1990
Fumagillin analogs (angiohibins)	Endothelial cell proliferation	Maione, 1991 Ingber, 1990
Herbimycin A	Endothelial cell proliferation	Shibata, 1986 Oikawa, 1989
GPA1734	Collagen synthesis	Missirlis, 1990
Somatostatin analogs	Unknown	Zalatnai, 1989 Woltering, 1991

depletion (Brem, 1990; Gross, 1991). Some of these interfere with various steps in the angiogenic process and others may inhibit one or more of the numerous other growth factors and cytokines that act in addition to bFGF to induce and control angiogenesis. Taken together, these studies provide strong support and great promise for the pursuit of antiangiogenics as novel therapeutic agents.

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